The discovery of the exciplex-forming antibody was fortuitous in that immunization and in vivo selection were based solely on binding to the stilbene antigen, not on activation of emission. Methods that allow direct selection of luminescent scFvs—for example, by flow cytometry (*11*)—would be more efficient and might even allow discrimination between normal fluorogenic and exciplex-forming scFvs as a result of the different emission properties expected for such protein-dye complexes.

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GENOMICS

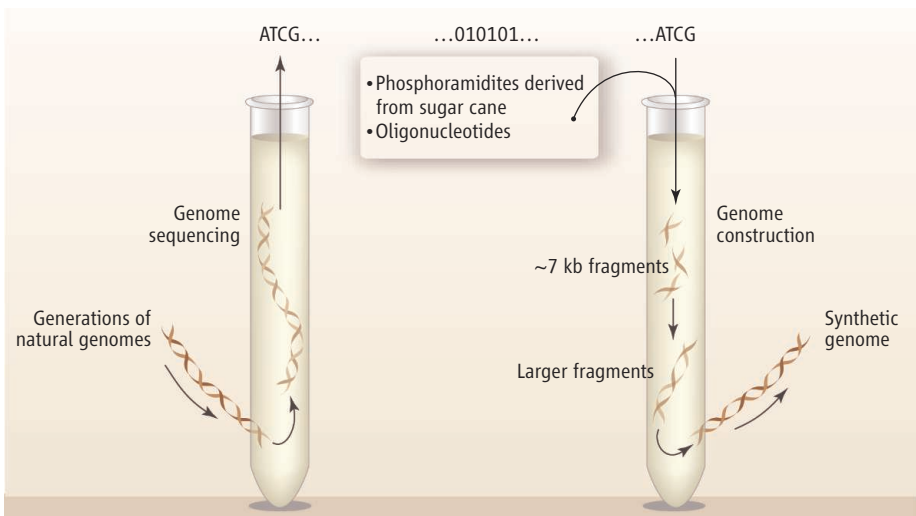
Reconstruction of the Genomes

Advances in DNA sequencing and synthesis technologies are making it possible to read and write entire genomes.

Drew Endy

"I am the family face; flesh perishes, I live on, projecting trait and trace through time to times anon, and leaping from place to place over oblivion." So starts the poem *Heredity* by Thomas Hardy, whose protagonist personifies the observation that all life exists through a process of direct descent from one generation to the next. Scientifically, the replication and propagation of genetic material, as DNA or RNA, is the primary mechanism by which each generation transmits the instructions underlying the traits and traces of their offspring. On page 1215 of this issue, Gibson *et al*. (*1*) bypass nature's constraint of direct descent by combining information and raw chemicals to construct the entire set of genetic material, or genome, encoding a bacterium (see the figure). This first construction of a genome encoding a self-reproducing organism heralds important opportunities in both genetics and biotechnology, highlights the need for improved DNA construction technology, and reinforces the value of ongoing public discussion of the impacts of making organisms easier to engineer.

Gibson *et al*. used a multistage process to construct the genome of *Mycoplasma genitalium*. First, information defining the 582,970–base pair (bp) DNA sequence of the genome to be synthesized was obtained from a computer database and divided into shorter sections, or cassettes of DNA up to ~7000 bp long. Commercial DNA suppliers then constructed these cassettes. Raw chemicals derived from sugar cane were combined to synthesize specific oligonucleotides, short fragments of DNA up to several hundred base pairs long (*2*). The suppliers then combined subsets of oligonucleotides to produce the requested cassettes (*3*). Gibson *et al*. used a hierarchical scheme to assemble, check, and, as needed, repair ever-longer DNA fragments, eventually producing the full-length genome.



Genome construction. DNA sequencing technology decodes the genome of an organism. DNA synthesis and genome construction technologies enable the opposite process. Bacterial genomes can be built from DNA sequence information and raw chemicals.

Given that all life is encoded by genetic material, ongoing and future advances in DNA synthesis and genome construction technology will be important. For example, the U.S. National Institutes of Health is estimated to spend ~$1.5 billion annually supporting the manual manipulation of DNA (*4*). Such work consumes most of the experimental effort for many biologists and biological engineers, a hidden opportunity cost that is harder to quantify. Moreover, the required slavish mastery of ad hoc methods and tedious tools for DNA manipulation discourages most students and researchers in fields such as physics, electrical engineering, and computer science from exploring biomedical and biotechnology research. Thus, an improved ability to provide any DNA molecule quickly, reliably, and economically would enhance and expand life sciences and engineering research (*5*), and might well become the goal of well-coordinated public research programs. Unfortunately, no such programs exist today.

Meanwhile, consider that most early discoveries of genetically encoded functions depended on analysis of the linkage between natural or randomly generated mutations and phenotypes (*6*), a powerful approach akin to blindly smashing many cars with a hammer and then determining which broken parts matter by attempting to drive each machine. Over the past 30 years, the invention (*7*) and development (*8*) of DNA sequencing technology have provided a complementary approach for discovering genetic functions. By comparing DNA sequence information from different organisms, researchers can

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now identify sequences that have remained relatively constant throughout millions of years of evolution (*9*). The presence of a DNA sequence across distantly related organisms implies that disruption of the sequence via an evolutionary "hammer" would have produced a deleterious effect on the organism, and thus the conserved sequence likely encodes an important function.

However, two additional approaches are needed to confirm and exhaustively identify all functions encoded by a natural DNA sequence. Specific DNA sequences thought to affect phenotypes must be purposefully changed and the expected effect confirmed. Also, seemingly irrelevant DNA sequences must be removed, disrupted, or otherwise modified and shown to be unnecessary. To date, the application of these additional approaches has been limited to short DNA sequences (*10*) or well-studied organisms (*11*). In developing their genome construction methods, Gibson *et al.* are hoping to more readily explore whether genes that can be individually disrupted (*12*) might also be disrupted in combination. Going forward, the ability to implement many simultaneous and directed changes to natural DNA sequences (*13*) and to build and test synthetic systems (*14*) will give researchers a powerful new "hammer" for constructing how life works.

The 582,970-bp "synthetic" genome produced by Gibson *et al*. also unequivocally demonstrates that it is now possible to construct the genomes for all known human viruses, including strictly regulated pathogens (such as smallpox), from publicly available DNA sequence data, methods, and materials. For now, the process of genome construction, as well as the production of an infectious agent given a newly synthesized but inert genome, requires highly skilled experts and considerable resources (Gibson *et al*. must still demonstrate that their synthesized genome will encode a living bacterium). In the meantime, recent international efforts to establish and coordinate best safety and security practices among competing DNA suppliers can be celebrated and improved (*15*). And, new efforts might focus on developing professional societies and improved standards of practice among biological engineers.

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CHEMISTRY

Getting Specific About Specific Ion Effects

Recent studies are shedding light on the mechanisms that drive the properties of salt solutions.

Douglas J. Tobias and John C. Hemminger

Have you noticed that "lite" salt, which is a mixture of KCl and NaCl, tastes slightly different from ordinary table salt, which is essentially pure NaCl? If so, then you have experienced a specific ion effect. Such effects are ubiquitous in chemical and biochemical processes involving salt solutions and have traditionally been attributed to the influence of the salt ions on the structure of water. Yet, a surge of recent research has provided compelling evidence that we should instead think about these phenomena in terms of specific ion interactions with surfaces and influences on hydrophobic interactions (*1*–*5*).

In the 1880s, Hofmeister and co-workers investigated the relative ability of different salts to precipitate proteins from blood serum and egg whites (*6*). The work resulted in the following ranking for anions: $SO_4^{2-} > F^- > HPO_4^{2-} > CH_3COO^- > Cl^- > Br^- > NO_3^- > I^- > ClO_4^- > SCN^-$. Ions on the left side of this Hofmeister series salt out (precipitate) solutes, whereas ions on the right salt in (dissolve or denature) solutes. An analogous series can be constructed for cations. Similar trends have been found in many solution properties (*7*, *8*), including surface tensions, chromatographic selectivity, colloid stability, and protein denaturation temperatures. It is widely held that Hofmeister series reflect specific ion effects on the long-range structure of water: Ions on the left are structure makers, ions on the right structure breakers.

Two recent studies (*1*, *2*) mount a strong case against this structure maker/breaker concept. Smith *et al.* (*1*) analyzed Raman spectra of water OH vibrations in potassium halide solutions. The position of the band centers and the line shapes of OH vibrational spectra are sensitive to details of the hydrogen-bonding network. Spectra of fluoride solutions are slightly blue-shifted compared with neat water, whereas solutions of the heavier halides are red-shifted. These effects were previously explained in terms of the structure-making and -breaking abilities of these ions. Using Monte Carlo simulations, Smith *et al.* show that the different halide ions do produce spectroscopically distinct changes to water hydrogen bonding, but these perturbations are largely confined to the first solvation shell. This result is consistent with an earlier spectroscopic study of the dynamics of halide ion solvation shells (*9*).

Mancinelli *et al.* found contradictions in the structure maker/breaker concept in a neutron diffraction study of NaCl and KCl solutions (*2*). According to the conventional Hofmeister series for cations, both Na^+ and K^+ are water-structure breakers. Yet, the analysis of the diffraction data suggested that, whereas water molecules are more orientationally disordered around the K^+ ion, Na^+ is more tightly solvated and more disruptive to water-water correlations.

Clues to more accurate explanations for specific ion effects are emerging from studies of the behavior of ions near interfaces.

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